

USE OF 2-METHYLENE-19-NOR-20(S)-1 α ,25-DIHYDROXYVITAMIN D₃
TO INCREASE BONE STRENGTH

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USE OF 2-METHYLENE-19-NOR-20(S)-1 α ,25-DIHYDROXYVITAMIN D₃ TO INCREASE BONE STRENGTH

BACKGROUND OF THE INVENTION

5 This invention relates to vitamin D compounds, and more particularly to pharmaceutical uses for 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃.

The natural hormone, 1 α ,25-dihydroxyvitamin D₃ and its analog in ergocalciferol series, i.e. 1 α ,25-dihydroxyvitamin D₂ are known to be highly potent regulators of calcium homeostasis in animals and humans, and more recently their activity in cellular differentiation has been established, Ostrem et al., Proc. Natl. Acad. Sci. USA, 84, 2610 (1987). Many structural analogs of these metabolites have been prepared and tested, including 1 α -hydroxyvitamin D₃, 1 α -hydroxyvitamin D₂, various side chain homologated vitamins and fluorinated analogs. Some of these compounds exhibit an interesting separation of activities in cell differentiation and calcium regulation. This difference in activity may be useful in the treatment of a variety of diseases as renal osteodystrophy, vitamin D-resistant rickets, osteoporosis, psoriasis, and certain malignancies.

15 Recently, a new class of vitamin D analogs has been discovered, i.e. the so called 19-nor-vitamin D compounds, which are characterized by the replacement of the A-ring exocyclic methylene group (carbon 19), typical of the vitamin D system, by two hydrogen atoms. Biological testing of such 19-nor-analogs (e.g., 1 α ,25-dihydroxy-19-nor-vitamin D₃) revealed a selective activity profile with high potency in inducing cellular differentiation, and very low calcium mobilizing activity. Thus, these compounds are potentially useful as therapeutic agents for the treatment of malignancies, or the treatment of various skin disorders. Two different methods of synthesis of such 19-nor-vitamin D analogs

have been described (Perlman et al., Tetrahedron Lett. 31, 1823 (1990); Perlman et al., Tetrahedron Lett. 32, 7663 (1991), and DeLuca et al., U.S. Pat. No. 5,086,191).

In U.S. Pat. No. 4,666,634, 2 β -hydroxy and alkoxy (e.g., ED-71) analogs
5 of 1 α ,25-dihydroxyvitamin D₃ have been described and examined by Chugai group as potential drugs for osteoporosis and as antitumor agents. See also Okano et al., Biochem. Biophys. Res. Commun. 163, 1444 (1989). Other 2-substituted (with hydroxyalkyl, e.g., ED-120, and fluoroalkyl groups) A-ring analogs of 1 α ,25-dihydroxyvitamin D₃ have also been prepared and tested
10 (Miyamoto et al., Chem. Pharm. Bull. 41, 1111 (1993); Nishii et al., Osteoporosis Int. Suppl. 1, 190 (1993); Posner et al., J. Org. Chem. 59, 7855 (1994), and J. Org. Chem. 60, 4617 (1995)).

Recently, 2-substituted analogs of 1 α ,25-dihydroxy-19-nor-vitamin D₃ have also been synthesized, i.e. compounds substituted at 2-position with hydroxy or
15 alkoxy groups (DeLuca et al., U.S. Pat. No. 5,536,713), which exhibit interesting and selective activity profiles. All these studies indicate that binding sites in vitamin D receptors can accommodate different substituents at C-2 in the synthesized vitamin D analogs.

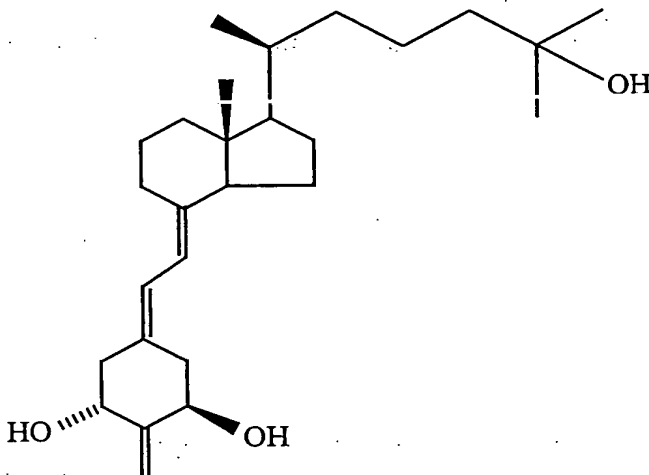
In a continuing effort to explore the 19-nor class of pharmacologically
20 important vitamin D compounds, an analog which is characterized by the presence of a methylene substituent at the carbon 2 (C-2) has been synthesized and tested. Of particular interest is the analog which is characterized by the unnatural configuration of the methyl group at carbon 20 (C-20), i.e. 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃. This vitamin D analog seemed an
25 interesting target because the relatively small methylene group at C-2 should not interfere with the vitamin D receptor. Moreover, molecular mechanics studies performed on the model 1 α -hydroxy-2-methylene-19-nor-vitamins indicate that

such molecular modification does not change substantially the conformation of the cyclohexanediol ring A. However, introduction of the 2-methylene group into 19-nor-vitamin D carbon skeleton changes the character of its 1 α - and 3 β - A-ring hydroxyls. Both hydroxyls are allylic to the exocyclic methylene group similar to the 1 α -hydroxyl group (crucial for biological activity) in the molecule of the natural hormone, 1 α ,25-(OH) $_2$ D $_3$.

SUMMARY OF THE INVENTION

The present invention is directed toward 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D $_3$, its biological activity, and various pharmaceutical uses for this compound.

Structurally this 19-nor analog is characterized by the general formula I shown below:



The solid wedge-shaped line to the methyl substituent at C-20 indicates that carbon 20 has the S configuration.

The above compound exhibits a desired, and highly advantageous, pattern of biological activity. This compound is characterized by intestinal calcium transport activity, similar to that of 1 α ,25-dihydroxyvitamin D $_3$, but exhibiting very

high activity, as compared to $1\alpha,25$ -dihydroxyvitamin D_3 , in its ability to mobilize calcium from bone. Hence, this compound is highly specific in its calcemic activity. Its preferential activity on mobilizing calcium from bone allows the in vivo administration of this compound for the treatment of metabolic bone diseases where bone loss is a major concern. Because of its preferential activity on bone, this compound would be a preferred therapeutic agent for the treatment of diseases where bone formation is desired, such as osteoporosis, especially low bone turnover osteoporosis, steroid induced osteoporosis, senile osteoporosis or postmenopausal osteoporosis, as well as osteomalacia and renal osteodystrophy. The treatment may be transdermal, oral or parenteral. The compound may be present in a composition in an amount from about $0.1\mu\text{g/gm}$ to about $50\mu\text{g/gm}$ of the composition, and may be administered in dosages of from about $0.1\mu\text{g/day}$ to about $10\mu\text{g/day}$.

The compound of the invention is also especially suited for treatment and prophylaxis of human disorders which are characterized by an imbalance in the immune system, e.g. in autoimmune diseases, including multiple sclerosis, diabetes mellitus, host versus graft reaction, and rejection of transplants; and additionally for the treatment of inflammatory diseases, such as rheumatoid arthritis and asthma, as well as the improvement of bone fracture healing and improved bone grafts. Acne, alopecia, skin conditions such as dry skin (lack of dermal hydration), undue skin slackness (insufficient skin firmness), insufficient sebum secretion and wrinkles, and hypertension are other conditions which may be treated with the compound of the invention.

The above compound is also characterized by high cell differentiation activity. Thus, this compound also provides a therapeutic agent for the treatment of psoriasis, or as an anti-cancer agent, especially against leukemia, colon cancer, breast cancer and prostate cancer. The compound may be present in a composition to treat psoriasis in an amount from about $0.01\mu\text{g/gm}$ to about

50 μ g/gm of the composition, and may be administered topically, transdermally, orally or parenterally in dosages of from about 0.01 μ g/day to about 10 μ g/day.

It has also been discovered that this compound increases breaking strength (cortical strength) as well as crushing strength (trabecular strength) of bones. Thus, this compound could also be used in conjunction with bone replacement procedures such as hip replacements, knee replacements, and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph illustrating the relative activity of 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃, 2-methylene-19-nor-1 α ,25-dihydroxyvitamin D₃ and 1 α ,25-dihydroxyvitamin D₃ to compete for binding of [³H]-1,25-(OH)₂-D₃ to the vitamin D pig intestinal nuclear receptor;

Figure 2 is a graph illustrating the intestinal calcium transport activity of 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ as compared to 1 α ,25-dihydroxyvitamin D₃;

Figure 3 is a graph illustrating the bone calcium mobilization activity of 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ as compared to 1 α ,25-dihydroxyvitamin D₃;

Figure 4 is a graph illustrating the bone mineral density in ovariectomized old female rats as a result of treatment with 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ as compared to 1 α ,25-dihydroxyvitamin D₃;

Figure 5 is a graph illustrating the percent HL-60 cell differentiation as a function of the concentration of 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃, 2-methylene-19-nor-1 α ,25-dihydroxyvitamin D₃ and 1 α ,25-dihydroxyvitamin D₃;

Figure 6a is a bar graph illustrating the restoration and building of bone in ovariectomized old female rats as a result of treatment with 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ as compared to 1 α ,25-dihydroxyvitamin D₃;

Figure 6b is a bar graph illustrating the increase of bone strength in ovariectomized old female rats as a result of treatment with 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ as compared to 1 α ,25-dihydroxyvitamin D₃;

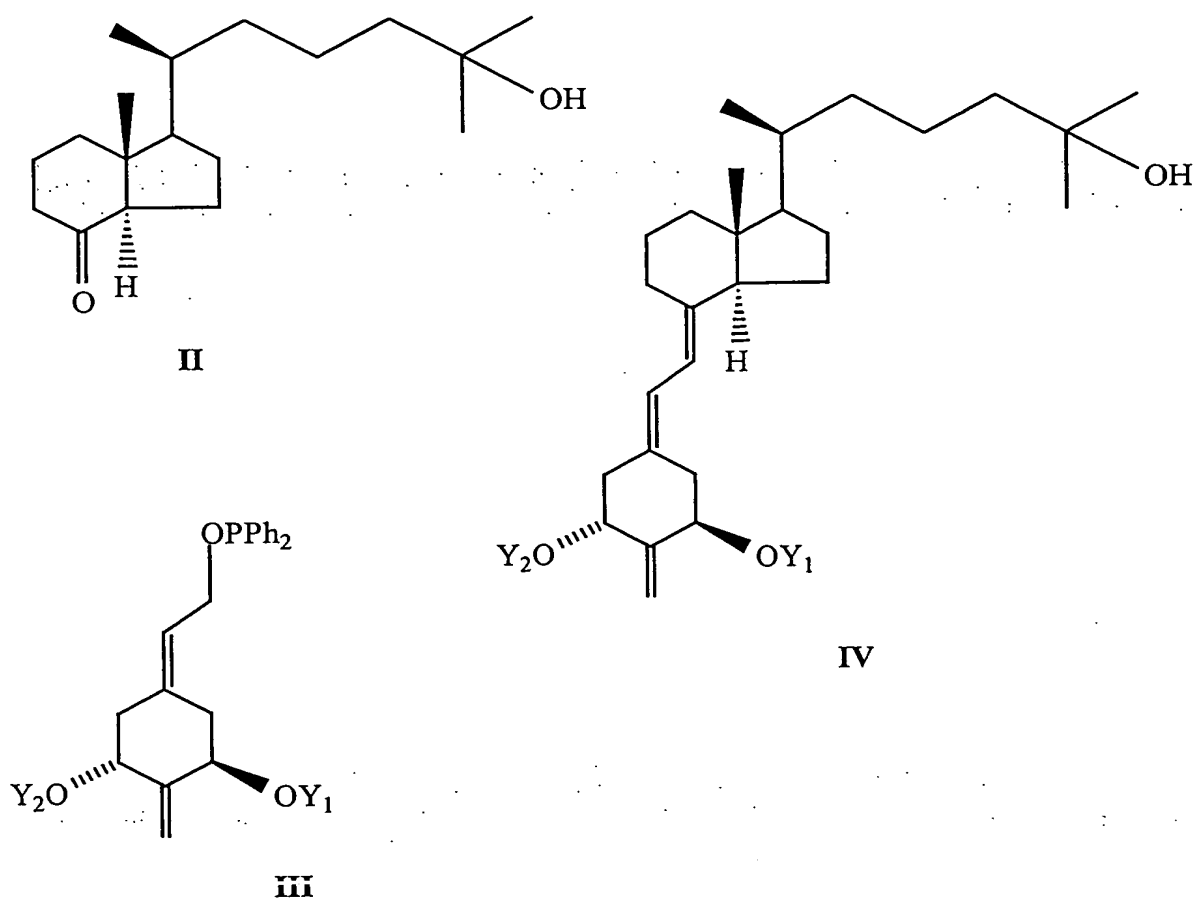
Figure 7 is a bar graph illustrating blood serum calcium levels in ovariectomized old female rats after 6 weeks of treatment at various dosages of 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ as compared to 1 α ,25-dihydroxyvitamin D₃; and

Figure 8 is a graph illustrating the growth of ovariectomized old female rats at various dosages of 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ as compared to 1 α ,25-dihydroxyvitamin D₃.

DETAILED DESCRIPTION OF THE INVENTION

2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ (referred to herein as 2MD) was synthesized and tested. Structurally, this 19-nor analog is characterized by the general formula I previously illustrated herein.

The preparation of 2-methylene-19-nor-20(S)-1 α ,25-dihydroxyvitamin D₃ having the basic structure I can be accomplished by a common general method, i.e. the condensation of a bicyclic Windaus-Grundmann type ketone II with the allylic phosphine oxide III to the corresponding 2-methylene-19-nor-vitamin D analog IV followed by deprotection at C-1 and C-3 in the latter compound:



In the structures II, III, and IV groups Y₁ and Y₂ are hydroxy-protecting groups, it being also understood that any functionalities that might be sensitive, or that
 5 interfere with the condensation reaction, be suitably protected as is well-known in the art. The process shown above represents an application of the convergent synthesis concept, which has been applied effectively for the preparation of vitamin D compounds [e.g. Lythgoe et al., J. Chem. Soc. Perkin Trans. I, 590 (1978); Lythgoe, Chem. Soc. Rev. 9, 449 (1983); Toh et al., J. Org. Chem. 48,
 10 1414 (1983); Baggiolini et al., J. Org. Chem. 51, 3098 (1986); Sardina et al., J. Org. Chem. 51, 1264 (1986); J. Org. Chem. 51, 1269 (1986); DeLuca et al., U.S. Pat. No. 5,086,191; DeLuca et al., U.S. Pat. No. 5,536,713].

Hydrindanones of the general structure II are known, or can be prepared by known methods.

For the preparation of the required phosphine oxides of general structure III, a new synthetic route has been developed starting from a methyl quinate derivative which is easily obtained from commercial (1R,3R,4S,5R)-(-)-quinic acid as described by Perlman et al., Tetrahedron Lett. 32, 7663 (1991) and DeLuca et al., U.S. Pat. No. 5,086,191.

The overall process of the synthesis of compound I is illustrated and described more completely in U.S. Patent No. 5,843,928 issued December 1, 1998 and entitled "2-Alkylidene-19-Nor-Vitamin D Compounds" the specification of which is specifically incorporated herein by reference.

BIOLOGICAL ACTIVITY OF 2-METHYLENE-20(S)- 19-NOR-1,25-(OH)₂D₃

The introduction of a methylene group to the 2-position of the 20(S) isomer of 19-nor-1,25-(OH)₂D₃ had little or no effect on binding to the porcine intestinal vitamin D receptor. This compound bound equally well to the porcine receptor as compared to the standard 1,25-(OH)₂D₃ (Figure 1). It might be expected from these results that this compound would have equivalent biological activity. Surprisingly, however, the 2 methylene and 20(S) substitutions produced a highly selective analog with its primary action on bone.

Figure 2 shows that 2MD has activity similar to that of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), the natural hormone, in stimulating intestinal calcium transport.

Figure 3 clearly demonstrates that 2MD is 100 times more potent than 1,25(OH)₂D₃ on bone, i.e. the mobilization of bone calcium.

Figure 4 shows that 2MD is extraordinarily effective in building bone mass in ovariectomized rats as compared to the native hormone without increasing serum calcium concentration. This is as yet an unprecedented new finding for a vitamin D compound.

5 Figure 5 illustrates that 2MD is 50-100 times more potent than 1,25(OH)₂D₃ on HL-60 differentiation, making it an excellent candidate for the treatment of psoriasis and cancer, especially against leukemia, colon cancer, breast cancer and prostate cancer.

10 Table 1 and Figure 6a illustrate that 2MD is very effective in restoring bone of ovariectomized, old female rats at 32 pmol given 2 times per week as compared to 1,25(OH)₂D₃ given at high doses 3 times per week. Note: 2MD also increases % ash in the femur.

15 Table 2 and Figure 6b show that 2MD increases breaking strength in the femurs (cortical strength) and crushing strength in the vertebra (trabecular strength) of animals shown in Table 1.

Figures 7 and 8 show a six week toxicity study in rats showing that 2MD appears safe at up to 30 pmol/day. Further, in Rhesus monkeys, a single oral dose of 27 µg does not cause significant elevation of serum calcium concentration, suggesting even greater safety in primates.

20 Competitive binding of the analogs to the porcine intestinal receptor was carried out by the method described by Dame et al (Biochemistry 25, 4523-4534, 1986).

The differentiation of HL-60 promyelocytic into monocytes was determined as described by Ostrem et al (J. Biol. Chem. 262, 14164-14171, 25 1987).

INTERPRETATION OF DATA

The in vivo tests of increasing serum calcium of rats on a zero calcium diet provides an insight to osteoblastic or bone activity of 2MD. The dose

response curves show that 2MD is at least 80 times more potent than 1,25(OH)₂D₃ in raising calcium in the plasma via the stimulation of the osteoblasts (Figure 3). At the same time, the activity of 2MD on intestinal calcium transport is approximately equal that of 1,25-(OH)₂D₃ (Figure 2). Therefore, these data show 2MD to have selective activity on bone.

2MD is about as active as 1,25(OH)₂D₃ in binding to the vitamin D receptor (Figure 1). However, it is between 10-100 times more active than 1,25-(OH)₂D₃ in causing differentiation of the promyelocyte, HL-60, into the monocyte (Figure 5). This result suggests that 2MD will be very effective in psoriasis because it has direct cellular activity in causing differentiation and in suppressing growth. It also indicates that it will have significant activity as an anti-cancer agent, especially against leukemia, colon cancer, breast cancer and prostate cancer.

The most important result, however, is that 2MD is extremely effective not only in restoring bone mass of ovariectomized, old female breeder rats as shown in Figures 4 and 6 and Tables 1 and 2, but it causes an increase in bone mass above that of sham-operated controls. This illustrates that 2MD is very likely having an anabolic effect on bone or increasing bone formation. Importantly, the increased bone mass provided by 2MD translates into marked increases in bone strength. This increased strength to fracture in femur shows cortical strength while increased strength to crush fractures of vertebra illustrates trabecular bone strength (Table 2 and Figures 6a and 6b). Interestingly, even the percent ash is unexpectedly increased further by 2MD. Of great importance is that at the dosage levels used in this study, there was no change in serum calcium of animals that showed the marked elevation of bone mass. This argues that a window of safety exists between the use of 2MD to increase bone mineral content and the action of 2MD in elevating serum calcium.

Preliminary safety tests carried out on two different occasions have revealed that female rats on a high calcium chow diet tolerate 30 pmol/day without elevating serum calcium or causing mineralization of the kidney (see Figs. 7 and 8). Further, preliminary studies in Rhesus monkeys indicates that primates tolerate 2MD extremely well since a dose of as much as 27µg of this compound was given as a single does to a 10 Kg Rhesus monkey without appreciably elevating serum calcium concentration. These and other tests indicate that primates will tolerate 2MD extremely well which may give a very large window between efficacy and the danger of hypercalcemia in man.

These results illustrate that 2MD is an excellent candidate for an anti-osteoporosis therapy and that it may be useful in a number of other circumstances such as autoimmune diseases, cancer, and psoriasis.

Table 1

Treatment of Ovariectomized Rats with 1,25-(OH)₂D₃ and 2MD

Group	Treatment	Treatment Time (Weeks)	BMD (g/cm ²)	BMC (g)	Body Wt. (g)	BMC/Body Wt. (mg/g)	Serum CA (mg/dl)	Femur Ash (%)	Femur Ash (mg)
OVX Control	Oil Vehicle/5X/Week	8	0.294±0.004	8.64±3.30	414±15	21.4±1.20	---	---	---
		17	0.296±0.003	9.34±0.50	422±19	22.3±1.69	---	---	---
		30	0.296±0.003	9.41±0.45	404±24	23.4±1.60	11.1±0.17	59.2±0.82	386±21.6
Sham Operated	Oil Vehicle/5X/Week	8	0.302±0.003	9.34±0.38	356±14	26.3±0.76			
		17	0.300±0.002	9.14±0.54	351±15	26.4±0.82			
		30	0.297±0.004	9.20±0.53	340±13	26.7±1.20	11.8±0.20	81.5±1.20	400±18.0
1,25(OH) ₂ D ₃	250 pmol/d/5X/Week	8	0.297±0.001	8.90±0.40	399±9.3	22.4±0.48			
		17	0.308±0.003	9.6±0.39	394±11	24.5±0.87			
		30	0.310±0.007	10.1±0.30	392±16	26.1±0.97	11.4±0.21	60.8±1.1	417±23
1,25(OH) ₂ D ₃	500 pmol/d/5X/Week 3X/Week 3X/Week	8	0.312±0.005	10.2±0.40	397±14.2	26.3±0.57			
		17	0.331±0.003	11.5±0.25	421±12.8	27.6±0.68			
		30	0.328±0.003	11.8±0.23	432±23.0	28.0±0.69	11.9±0.20	61.4±1.3	478±7.5
2MD	32 pmol/d/2X/Week	8	0.295±0.003	8.4±0.13	375±8.2	22.4±0.64			
		17	0.313±0.011	9.7±0.19	373±11.0	26.2±0.92			
		30	0.331±0.003	11.6±0.40	346±11.0	33.4±1.60	10.8±0.22	65.6±1.7	462±21.4
2MD	65 pmol/d/1X/Week	8	0.293±0.004	8.5±0.23	408±10.5	22.2±0.53			
		17	0.312±0.005	9.6±0.24	402±11.3	24.0±0.80			
		30	0.310±0.003	10.2±0.33	393±15.0	26.0±1.10	10.7±0.46	62.5±0.57	443±11.6

All animals were ovariectomized except the sham-operated controls. Values are expressed as mean ± SEM.

Table 2

Strength of Femurs and Vertebrae to Mechanical Stress

Group	Treatment	Stress Value Femur	Stress Value Vertebra
OVX Control	Oil Vehicle/5X/Week	109.31±19.60	14.26±3.58
Sham-Operated	Oil Vehicle/5X/Week	121.36±12.5	13.67±1.79
1,25(OH) ₂ D ₃	250 pmol/day/5X/Week	118.21±19.85	19.24±5.66
1,25(OH) ₂ D ₃	500 pmol/d/3-5X/Week	116.47±16.20	17.14±0.52
2MD	32 pmol/d/2X/Week	134.84±14.12	23.93±6.59
2MD	65 pmol/d/1X/Week	133.71±14.06	17.07±5.73

5 For treatment purposes, the compound of this invention defined by formula I may be formulated for pharmaceutical applications as a solution in innocuous solvents, or as an emulsion, suspension or dispersion in suitable solvents or carriers, or as pills, tablets or capsules, together with solid carriers, according to conventional methods known in the art. Any such formulations may
10 also contain other pharmaceutically-acceptable and non-toxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents.

The compound may be administered orally, topically, parenterally or transdermally. The compound is advantageously administered by injection or by
15 intravenous infusion or suitable sterile solutions, or in the form of liquid or solid doses via the alimentary canal, or in the form of creams, ointments, patches, or similar vehicles suitable for transdermal applications. Doses of from 0.1 µg to 10 µg per day of the compounds are appropriate for treatment purposes, such doses being adjusted according to the disease to be treated, its severity and the
20 response of the subject as is well understood in the art. Since the compound exhibits specificity of action, each may be suitably administered alone, or together with graded doses of another active vitamin D compound -- e.g. 1α-

hydroxyvitamin D₂ or D₃, or 1 α ,25-dihydroxyvitamin D₃ -- in situations where different degrees of bone mineral mobilization and calcium transport stimulation is found to be advantageous.

5 Compositions for use in the above-mentioned treatment of psoriasis and other malignancies comprise an effective amount of the 2-methylene-20(S)-19-nor-vitamin D compound as defined by the above formula I as the active ingredient, and a suitable carrier. An effective amount of such compound for use in accordance with this invention is from about 0.01 μ g to about 50 μ g per gm of composition, and may be administered topically, transdermally, orally or
10 parenterally in dosages of from about 0.1 μ g/day to about 10 μ g/day.

The compound may be formulated as creams, lotions, ointments, topical patches, pills, capsules or tablets, or in liquid form as solutions, emulsions, dispersions, or suspensions in pharmaceutically innocuous and acceptable solvent or oils, and such preparations may contain in addition other
15 pharmaceutically innocuous or beneficial components, such as stabilizers, antioxidants, emulsifiers, coloring agents, binders or taste-modifying agents.

The compound is advantageously administered in amounts sufficient to effect the differentiation of promyelocytes to normal macrophages. Dosages as described above are suitable, it being understood that the amounts given are to
20 be adjusted in accordance with the severity of the disease, and the condition and response of the subject as is well understood in the art.

The formulations of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other therapeutic ingredients. The carrier must be "acceptable" in the sense of
25 being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof.

Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each

containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion.

5 Formulations for rectal administration may be in the form of a suppository incorporating the active ingredient and carrier such as cocoa butter, or in the form of an enema.

 Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably
10 isotonic with the blood of the recipient.

 Formulations suitable for topical administration include liquid or semi-liquid preparations such as liniments, lotions, applicants, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes; or solutions or suspensions such as drops; or as sprays.

15 For asthma treatment, inhalation of powder, self-propelling or spray formulations, dispensed with a spray can, a nebulizer or an atomizer can be used. The formulations, when dispensed, preferably have a particle size in the range of 10 to 100 μ .

 The formulations may conveniently be presented in dosage unit form and
20 may be prepared by any of the methods well known in the art of pharmacy. By the term "dosage unit" is meant a unitary, i.e. a single dose which is capable of being administered to a patient as a physically and chemically stable unit dose comprising either the active ingredient as such or a mixture of it with solid or liquid pharmaceutical diluents or carriers.